UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/082,661	02/20/2002	Kerry Kulowski	1221.001US1	6625

7590 09/02/2003

Mark A. Litman & Associates, P.A.
3209 West 76th St., Suite 205
Edina, MN 55435

EXAMINER
EPPERSON, JON D

ART UNIT	PAPER NUMBER
1639	5

DATE MAILED: 09/02/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/082,661	Applicant(s) KULOWSKI ET AL.
File Copy	Examiner Jon D Epperson	Art Unit 1639

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19* is/are pending in the application.

4a) Of the above claim(s) *10-16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9 and 17-19* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *2* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Status of the Application

1. Receipt is acknowledged of a Response to a Restriction Requirement, which was dated on June 4, 2003 (Paper No. 4).

Priority Claims

2. No foreign or domestic priority is claimed. Therefore, the effective filing date of the claims is the filing date of the case i.e., February 20, 2002.

Status of the Claims

3. Claims 1-18 were pending in the present application. Applicant added claim 19 in Paper No. 4. Therefore, claims 1-19 are currently pending.

4. Applicant's response to the Restriction and/or Election of Species requirements in Paper No. 4 is acknowledged (Applicant elected ***without traverse*** Group I, claims 1-9 and 17-19) and claims 10-16 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected inventions, there being no allowable generic or linking claim.

5. Please note: Applicant's elected species (Host Cell = Streptomyces lividans; Biotransformation Gene = PikC; Number of Biotransformation Genes = 3; Chemical

Substrate = narbomycin; biocatalyst = monoxygenase (pikC enzyme); Chemical functional Group = pikromycin; reaction = hydroxylation; functional group added = hydroxyl; method steps = tranformation of streptomyces protoplasts) was found in the art. Applicant is reminded of MPEP § 803.02 with respect to species elections:

> On the other hand, should no prior art be found that anticipates or renders obvious the elected species, the search of the Markush-type claim will be extended. If prior art is then found that anticipates or renders obvious the Markush-type claim with respect to a nonelected species, the Markush-type claim shall be rejected and claims to the nonelected species held withdrawn from further consideration. ***The prior art search, however, will not be extended unnecessarily to cover all nonelected species.*** Should applicant, in response to this rejection of the Markush-type claim, overcome the rejection, as by amending the Markush-type claim to exclude the species anticipated or rendered obvious by the prior art, the amended Markush-type claim will be reexamined. The prior art search will be extended to the extent necessary to determine patentability of the Markush-type claim. In the event prior art is found during the reexamination that anticipates or renders obvious the amended Markush-type claim, the claim will be rejected and the action made final. Amendments submitted after the final rejection further restricting the scope of the claim may be denied entry.

6. Therefore, claims 1-9 and 17-19 are examined on the merits in this action.

Response to Restriction and/or Election of Species

7. Applicant's election of Group I (claims 1-9 and 17-19) **without traverse** in Paper No. 4 is acknowledged.

8. Applicant's election of species in Paper No. 4 is also acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election of species has also been treated as an election without traverse (MPEP § 818.03(a) and/ or 37 CFR 1.111(b)).

9. As a result, the restriction requirement and/or election of species is still deemed proper and is therefore made FINAL.

Information Disclosure Statement

10. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98 (b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on the form PTO-892, they have not been considered.

11. The references listed on applicant's PTO-1449 form have been considered by the Examiner. A copy of the form is attached to this Office Action.

Specification

12. The specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

Objections to the Claims

13. Claim 4 is objected to because of the following informalities:

A. Claim 4 is missing a period. Appropriate correction is required.

B. Claim 1 states, "comprising a biocatalysts." It should read "comprising a biocatalyst" i.e., the "s" needs to be removed.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

14. Claims 1-9 and 17-19 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection.

Claims 1-9 and 17-19 are drawn to a genus of methods for producing "biocatalysts" using "host cells" that contain at least two "biotransformation genes" that are capable of producing "biocatalysts" that can modify "substrates" inside the host cells. The scope of these claims include an infinite number of methods for producing an infinite number of recombinant host cells (i.e., host cells with an infinite number of different "biotransformation genes") that will generate an infinite number of "biocatalysts" that will react with an infinite number of "substrates" to produce an infinite number of products wherein no distinguishing structural attributes are provided for the members of either the

biotransformation genes, biocatalysts, substrates or products. The specification and claims do not place any limit on the number of atoms, the types of atoms, or the manner in which said atoms might be connected to form the biotransformation genes, biocatalysts, substrates or products.

Although the specification provides one "hypothetical" example of a monoterpene substrate reacted with host cells that contain p450/o-methyltransferase plasmid constructs and also a "laundry list" of other "potential" substrates and enzymes (e.g., see specification, pages 10-11), the specification and claims do not provide ***any*** guidance as to what structural features ***all*** of these biotransformation genes, biocatalysts, substrates and products. Consequently, it is not possible to determine *a priori* which compounds the would be encompassed by Applicants' broad claims because there is no common structural attributes that can link together ***all*** of these potential biotransformation genes, biocatalysts, substrates and products i.e., there is no teaching that would allow a person of skill in the art to determine *a priori* ***all*** the different types of compounds that should be included in this broad genus from the ONE "working" example provide by applicant (i.e., the use of p450/o-methyltransferase with a monoterpene) (the word "working" is placed in quotations because Applicants do not actually characterize any of the products here, it is a complexly "hypothetical" example and thus NOT a true working example).

Thus, the specification provides only ONE "hypothetical" example (see specification, page 10, lines 27-28; see also page 11) for the biocatalysis of a monoterpene. One representative species (i.e., biocatalysis of a monoterpene) is

not enough to show possession of a genus that would encompass an infinite number of possibilities i.e., ***any*** substrate using ***any*** enzyme to form ***any*** product.

With respect to adequate disclosure applicant is referred to the discussion in *University of California v. Eli Lilly and Co.* (U.S. Court of Appeals Federal Circuit (CAFC) 43 USPQ2d 1398 7/22/1997 Decided July 22, 1997; No. 96-1175) regarding disclosure. For adequate disclosure, like enablement, requires *representative examples* which provide reasonable assurance to one skilled in the art that the compounds falling within the scope both possess the alleged utility and additionally demonstrate that *applicant had possession of the full scope of the claimed invention.* See *In re Riat*_(CCPA 1964) 327 F2d 685, 140 USPQ 471; *In re Barr* (CCPA 1971) 444 F 2d 349, 151 USPQ 724 (for enablement) and University *of California v. Eli Lilly and Co* cited above (for disclosure). The more unpredictable the art the greater the showing required (e.g. by "representative examples") for both enablement and adequate disclosure.

Although several other biometabolic pathways are known in the art in addition to the "hypothetical" monoterpene biosynthesis example provided by applicant (see 35 USC 102 rejections below), a written description of all biometabolic pathways is still not possible. In order to achieve possession of these broad claims, all genes associated with every biometabolic enzyme would have to be known (i.e., characterized), expressable (i.e., cloned) and be able to be functionally expressed (i.e., active in new host), which is simply not the case. \

For example, even within the limited scope of the carotenoid biosynthetic pathway (which would be encompassed by Applicants' broad claims) all genes

encoding all biometabolic enzymes have not been achieved. For example, applicants cannot express zeaxanthin epoxidase from *Nicotiana* in *E.coli* because this prokaryotic host cannot provide reduced ferredoxin, which is needed for epoxide formation i.e., to be functionally expressed (see Sandmann G.; Albrecht, M.; Schnurr, G.; Knorzer, O.; Boger, P., "The biotechnological potential and design of novel carotenoids by gene combination in *Escherichia coli*" Trends. Biotechnol. **1999**, 17, 233-237, see especially 234, column 2, paragraph 1).

Furthermore, the possibility of expressing new enzymes (which would also be encompassed by Applicants' broad claims) is either hit or miss since it does not depend on any rational basis for experimentation i.e., molecular modeling, etc. Furthermore, this "hit or miss" technology may be subject to poor screening techniques for a desired mutant (see Schmidt-Danert, C.; Arnold, F. H. "Directed evolution of industrial enzymes" The International Business Communications Second International Symposium on Directed Evolution of Industrial Enzymes, September 1998, see section on "Screening technology") ("There was consensus among the participants that one critical phase of any directed-evolution experiment is deciding how to search for variants with the desired properties. For most practical problems, this search is both time consuming and expensive").

Therefore, applicants are not is possession of ***any*** host cell comprising ***any*** biotransformation genes that encode ***any*** biocatalyst for the production of ***any*** product. Applicants' claimed scope represents only an invitation to experiment

regarding possible biometabolic enzymes that might be clones and used for a given metabolic pathway.

Furthermore, Applicants employ only "functional language" to describe critical elements of their invention. With regard to the description requirement, Applicants' attention is directed to The Court of Appeals for the Federal Circuit which held that a "written description on an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." *University of California v. Eli Lilly and Co.*, 43 USPQ2d 1398, 1405 (1997), quoting *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original)[The claims at issue in *University of California v. Eli Lilly* defined the invention by function of the claimed DNA (encoding insulin)]. Here, Applicant's describe the "biocatalysts" only by what they can do i.e., catalyze an unspecified reaction. The CAFC held this sort of functional definition insufficient to adequately describe the claimed product.

Given this lack of description of the representative species encompassed by the genus of the claims and the "improper" use of functional language, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the inventions of claims 1-9 and 17-19.

15. Claims 1-9 and 17-19 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a library of hosts cells containing plasmids encoding cytP450I, OMT and cytP450II (see specification, page 12, Table 1), does not reasonably provide enablement for ***any*** host cell containing a library of ***any*** biotransformation genes encoding ***any*** set of biocatalysts to produce ***any*** products. The specification does not enable any person skilled in the art to which it pertains, or with which it most nearly connected, to make and use the invention commensurate in scope with these claims. This is an enablement rejection.

Despite knowledge in the art for pathways other than the biocatalytic conversion of a monoterpene (see 35 USC 102 rejections below), the specification fails to provide guidance regarding how to create a library of host cells with genes that encode for ***any*** biocatalyst associated with ***any*** biometabolic pathway. Therefore, the breadth of these claims is much larger than the scope enabled by the specification.

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue". Some of these factors may include, but are not limited to:

(1) the breadth of the claims;
(2) the nature of the invention;
(3) the state of the prior art;
(4) the level of one of ordinary skill;
(5) the level of predictability in the art;
(6) the amount of direction provided by the inventor;
(7) the existence of working examples; and

(8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

See *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

(1-2) The breadth of the claims and the nature of the invention: The claims are drawn to a broad genus. The scope of these claims include an infinite number of methods for producing an infinite number of recombinant host cells (i.e., host cells with an infinite number of different "biotransformation genes") that will generate an infinite number of "biocatalysts" that will react with an infinite number of "substrates" to produce an infinite number of products wherein no distinguishing structural attributes are provided for the members of either the biotransformation genes, biocatalysts, substrates or products. The specification and claims do not place any limit on the number of atoms, the types of atoms, or the manner in which said atoms might be connected to form the biotransformation genes, biocatalysts, substrates or products. Consequently, the nature of the invention cannot be fully determined because the invention has not been defined with particularity.

(3 and 5) The state of the prior art and the level of predictability in the art: Combinatorial biosynthesis is a new and highly unpredictable field that requires identification/characterization of the "modular biosynthetic enzymatic machinery." With the exception of polyketides and nonribosomally produced peptides and carbohydrates, this has not been done. For example, Taylor states that for the biosynthesis of epothione would require a mixed NRPS/PKS "biosynthetic enzymatic machinery." However, Taylor states that there are

currently "no examples of such an approach [in the literature]" and that while it may be "easy to imagine how novel epothione analogs could be generated" (much like Applicants' "hypothetical" example for making monoterpene derivatives), "[m]uch work remains to be done in elucidating the organization and structure of hybrid PKSs/NRPSs, however, before combinatorial biosynthesis with these systems can be undertaken" (emphasis added) (see Taylor, S. V. in "Handbook of Combinatorial Chemistry" Eds. Nicolaou, K. C.; Hanko, R.; Hartwig, W. Weinheim Germany: Wiley-VCH 2002, Vol. 2, page 1075, last paragraph).

Another example is the carotenoid biosynthetic pathway (which would be encompassed by Applicants' broad claims) wherein the "biocatalyst" zeaxanthine epoxidase cannot be expressed in *E. coli.* because this prokaryotic host cannot provide reduced ferredoxin, which is needed for epoxide formation i.e., to be functionally expressed (see Sandmann G.; Albrecht, M.; Schnurr, G.; Knorzer, O.; Boger, P., "The biotechnological potential and design of novel carotenoids by gene combination in *Escherichia coli*" *Trends. Biotechnol.* **1999**, 17, 233-237, see especially 234, column 2, paragraph 1).

Therefore, the Examiner contends that the level of predictability in the art is low.

(4) The level of one of ordinary skill: The level of skill required would be high, most likely at the Ph.D. level.

(6-7) The amount of direction provided by the inventor and the existence of working examples: Applicants have not provided a single working example etc.

Applicants present only one "hypothetical" example for the biosynthesis of monoterpene derivatives.

(8) The quantity of experimentation needed to make or use the invention base on the content of the disclosure: As a result of the broad and unpredictable nature of the invention and the lack of specific guidance from the specification, the Examiner contends that the quantity of experimentation needed to make and or use the invention would be great. Note that there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed. *In re Vaeck*, 947 F.2d 488, 496 & n.23, 20 USPQ2d 1438, 1445 * n.23 (Fed. Cir. 19991). In this case, Applicants have not provided any working examples that would teach this enormous genus that falls within a highly unpredictable art area. Therefore, it is deemed that further research of an unpredictable nature would be necessary to make or use the invention as claimed. Thus, due to the inadequacies of the instant disclosure one of ordinary skill would not have a reasonable expectation of success and the practice of the full scope of the invention would require undue experimentation.

Claims Rejections - 35 U.S.C. 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

16. Claims 1-9 and 17-19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. ***Claims 1,17*** are rejected because the "biocatalysts" in these claims is not defined with any chemical or physical characteristic, but only by functional properties i.e., their ability to catalyze a biochemical reaction. A claim to a material defined solely in terms of what it can do, or a property thereof, does not particularly point out the claimed invention. A person of skill in the art cannot immediately envision all the possible chemical structures for a peptide/protein with this function i.e., biocatalytic activity. Thus, the metes and bounds of the claimed invention cannot be determined. See *ex parte Pulvari* (POBA 1966) 157 USPQ 169. Therefore, claims 1,17 and all dependent claims are rejected under 35 U.S.C. § 112, second paragraph.

B. **Claim 2** recites the limitation "the at least two chemical functional groups" in lines 2-3. There is insufficient antecedent basis for this limitation in the claim. The Examiner recommends "the at least two different chemical functional groups." Therefore, claim 2 and all dependent claims are rejected under 35 USC 112, second paragraph.

C. For **claim 2**, the phrase "introduce two different chemical functional groups ... selected from hydroxylation, halogenation" is vague and indefinite. For example, "hydroxylation" is NOT a "chemical functional group" i.e., a "hydroxyl" group is a functional group, hydroxylation is a process by which a

"hydroxyl group" is introduced. Applicants are requested to clarify and/or correct. Therefore, claims 2 and all dependent claims are rejected under 35 U.S.C. 112, second paragraph.

D. **Claim 3** recites the limitation "said biotransformation genes" in line 1. There is insufficient antecedent basis for this limitation in the claim. The Examiner recommends "said at least two biotransformation genes." Therefore, claim 3 and all dependent claims are rejected under 35 USC 112, second paragraph.

E. ***Claims 5-8*** are rejected because the "biotransformation genes" in these claims are not defined with any chemical or physical characteristic, but only by functional properties i.e., their ability to provide "functional group addition of groups capable of providing catalysis for processes selected from the group consisting of acylation ... etc." A claim to a material defined solely in terms of what it can do, or a property thereof, does not particularly point out the claimed invention. A person of skill in the art cannot immediately envision all the possible chemical structures for a peptide/protein with this function i.e., biocatalytic activity. Thus, the metes and bounds of the claimed invention cannot be determined. See *ex parte Pulvari* (POBA 1966) 157 USPQ 169.

Furthermore, it is not clear what Applicants' are referring to here. Do the biotransformation genes encode proteins that catalyze the addition of functional groups that "can be" acted on by enzymes that catalyze reactions for acylation, glycosylation, amidation? Do the biotransformation genes encode the "acylation, glycosylation, amidation" enzymes themselves? Do the genes act as substrates

that are subsequently transformed into said functional group? Applicants intent here is simply not clear. Therefore, claims 5-8 and all dependent claims are rejected under 35 USC 112, second paragraph.

Claims Rejections - 35 U.S.C. 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in-
> (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effect under this subsection of a national application published under section 122(b) only if the international application designating the United States was published under Article 21(2)(a) of such treaty in the English language; or
> (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that a patent shall not be deemed filed in the United States for the purposes of this subsection based on the filing of an international application filed under the treaty defined in section 351(a).

17. Claims 1-9 are rejected under 35 U.S.C. 102(b) as being anticipated by Xue et al (Xue, Q.; Ashley, G.; Hutchinson, C. R.; Santi, D. V. "A multiplasmid approach to preparing large libraries of polketides" PNAS, **October 12, 1999**, 96(21), 11740-11745).

For ***claim 1***, Xue et al discloses a collection (library) of recombinant *Streptomyces lividans* (host cells) carrying various combinations of three plasmids (containing biotransformation genes) representing mutant and/or wild type including eryAI, eryAII and eryAIII genes encoding for modules 1-2, 3-4, and 5-6

of the DEBS 1, 2 and 3 modular proteins (biometabolic enzymes), respectively (see Xue et al, page 11741, figure 1, showing wild type and mutant forms of eryA genes and DEBS proteins; see also page 11743, table 1, showing genotype of plasmids containing DEBS genes that were combined to produce a library of host cells for the generation of new macrolactones i.e., see macrolactones in figure 3).

Xue et al discloses modifying the substrate propionyl-CoA in the recombinant host cells (see Xue et al, page 11741, figure 1 see also page 11743, second column, last paragraph) ("In one example, we prepared a single Cys-729 → Ala mutation at the KS1 domain of DEBS1 module 1. The inactive KS1 prevents propagation of the starter unit and permits introduction of exogenous synthetic diketide thiol esters into position 12 and 13 of the 14-membered macrolide product. The plasmid encoding the KS1 null allele of eryAI was introduced by cotransformation into *S. lividans* with the 1 eryAII mutant and 7 eryAIII mutants (Table 1) to provide ***16 transformants*** [each containing more than one mutation])."

Xue et al states, that "[b]y using this multiple plasmid approach, with X mutants of ORF 1, Y mutants of ORF 2 and Z mutants of ORF 3, along with the wild-type genes, for instance, a ***combinatorial library*** of (X+1) × (Y+1) × (Z+1) ***mutants*** plus the wild-type PKS could be created expeditiously" (see Xue et al, page 11740, bottom of last paragraph) (see also Xue et al, page 11742, second column, last paragraph, stating "[a] demonstration ***library*** composed of ***three single mutations*** in eryAI (module 2), one in eryAII (module 3), and seven in eryAIII (modules 5 or 6) as well as wild-type ORFs was created by using this

three-plasmid system"). Xue et al also mentions the use of DNA shuffling techniques to prepare larger libraries of polyketides (see Xue et al, page 11740, column 2 second to last paragraph, "[a]nother strategy for preparing large numbers of polyketides is by random digestion-religation leading to "mutagenesis" of the domains or modules of a mixture of PKS genes, including the refinements embodied in the DNA shuffling method").

Furthermore, each mutant is "operably controlled" under the direction of the actI promoter and actII-ORF4 gene (see Xue et al, page 11742, figure 2) for the production of modular enzymes (biometabolic) that are involved in the polyketide biosynthetic pathway and the enzymes in these constructs create new biometabolic pathways producing new macrolactone derivatives (i.e., the enzyme is isolated from a biometabolic pathway that is different from the biometabolic pathway of which it is a component in the host cell and the mutated gene is a chimera of genes from different metabolic pathways e.g., erythromycin and rapamycin biometabolic pathways). Therefore, Xue et al anticipates all of the limitations in claim 1.

For ***claim 2***, Xue et al discloses the introduction of different chemical groups including a ketone and a carbon-carbon double bond. The carbon-carbon double bond reads on Applicants' claims because it discloses the formation of a "carbon to carbon bond" (see Xue et al, figure 1B).

For ***claims 3-4***, Xue et al discloses many reactions including oxidation (e.g., see Xue et al, figure 1 B wherein a hydroxyl group is oxidized to a ketone).

For ***claim 5***, Xue et al discloses compound 21 (see Xue et al, page 11744, figure 3), which has an almost identical in structure to Applicants' elected narbonolide species and thus would be expected to undergo "glycosylation" as shown by Applicants' "Diagrammatic Description of Species Example of Claim 19" (see Paper No. 4). Xue et al also states that the "PKS libraries generated could be leveraged and expanded by introducing genes for tailoring enzymes that oxidize, methylate, acylate, or glycosylate the product of the PKS" (see Xue et al, page 11745, column 1). "When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not." *In re Spada*, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990). The Office does not have the facilities to make such a comparison and the burden is on the applicants to establish the difference. See *In re Best,* 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *Ex parte Gray*, 10 USPQ 2d 1922 1923 (PTO Bd. Pat. App. & Int.).

For ***claims 6-9***, Xue et al discloses Applicants' elected *Streptomyces lividans* host cells and both constitutive and inducible promoters to create whole cell biocatalysts (see Xue et al, page 11741-42, Materials and Methods; see also figure 2).

18. Claims 1-3 and 5-8 are rejected under 35 U.S.C. 102(b) as being anticipated by Stachelhaus et al (Stachelhaus, T.; Schneider, A.; Marahiel, M. A. "Rational Design of Peptide Antibiotics by Targeted Replacement of Bacterial and Fungal Domains" Science, **July 1995**, 269, 69-72).

For ***claim 1***, Stachelhaus et al discloses a collection (library) of *E. coli* and *B. subtilis* strains (host cells) carrying various chimeric peptide synthetase constructs (See Stachelhaus et al, page 72, table 1). Stachelhaus et al discloses recombining "at least two" biotransformation genes including srfA gene from *B. subtilis* with the Phe, Orn, and Leu activating domains from the grs operon from *Bacillus brevis* (replacing the Leu srfA-C from *B. subtilis*) to form a gene chimera from different organisms (see Stachelhaus et al, page 73, table 1; see also page 70, column 1, paragraph 3; see also figure 4 showing replacement of "at least two" biotransformation genes into each of the [Cys^7]surfactin "isoforms"). Furthermore, these chimeric peptide synthetase constructs encode for synthetases (i.e., biocatalysts) that are used to produce peptides by a nonribosomal mechanism and the enzyme in these constructs creates new biometabolic pathways producing new peptide derivatives (i.e., the enzyme is isolated from a biometabolic pathway that is different from the biometabolic pathway of which it is a component in the host cell and the mutated gene is a chimera of genes from different metabolic pathways), which anticipates claim 1 (see Stachelhaus et al, page 72, table 1; see also pages 70-71, figures 1-3).

For ***claim 2***, Stachelhaus et al discloses the addition of Cys, Phen, Orn, Leu and Val into the surfactin isoforms which would read on the introduction of carbon-carbon double bonds (see page 72, Table 1).

For ***claim 3***, Stachelhaus et al discloses replacement of leucine for Cys, Phe, Orn, Val (see page 72, Table 1).

For ***claim 5***, Stachelhaus et al discloses acylation or glycosylation (see page 72, column 2, paragraph 1).

For ***claim 6-8***, Stachelhaus et al discloses *E. coli* and *B. subtilis* (see Stachelhaus et al, page 72, table 1). Stachelhaus et al also discloses an srfA-C integration vector under the control of a heat inducible tandem P_r/P_l promoter (see page 70, figure 2) that was used to create the library of recombinant host cells with the expression vectors.

19. Claims 1-9 are rejected under 35 U.S.C. 102(b) as being anticipated by Albrecht et al (Albrecht, M.; Takaichi, S.; Misawa, N.; Schnurr, G.; Boger, P.; Sandmann, G. "Synthesis of atypical cyclic and acyclic hydroxy carotenoids in *Escherichia coli* transformants" *Journal of Biotechnology*, **1997**, 58, 177).

For ***claims 1-9***, Albrecht et al discloses a collection (library) of *Escherichia coli* strains (recombinant host cells) carrying various plasmids (biotransformation genes) mutated to contain various carotenoic genes e.g., crtE, crtB, crtI, etc (see Albrecht et al, page 178, table 1) encoding enzymes (biocatalysts) for the production of various terpenoids (see Albrecht et al, page 182, figure 3). Albrecht et al discloses *E. coli* host cells containing plasmids that have been mutated to carry more than one gene i.e., "at least one" (see Albrecht et al, page 178, table 1, e.g., pACCRT-EBI$_{Rc}$, pACCRT-EBI$_{Eu}$, etc). Furthermore, each vector mutated to express the terpenoid genes is "operably controlled" using different replicons (see Albrecht et al, page 178, table 1) for the production of

modular enzymes (biometabolic) that are involved in terpenoid biosynthesis (see Albrecht et al, abstract, "A total of eight different hydroxy carotenoids we reproduced in transformants of the non-carotenogenic bacterium Escherichia coli. They include the acyclic 1-hydroxyneurosporene, 1-hydroxylcopene, 1,1'-dihydroxylcopene, [etc]"), which reads on parts (a), (b) and (c) of claim 1 i.e., the mutated gene is a chimera of genes from different metabolic pathways (see for Albrecht et al, page 182, figure 3, for part (a) showing different metabolic pathways that produce different terpenoids via different chimeric enzymes) (see also Albrecht et al, abstract, for parts (b) and (c) showing carotenoids that are produced in "non-carotenogenic bacterium"). Therefore, Albrecht et al anticipates all of the limitations in claim 1.

20. Claims 1-9 and 17-19 are rejected under 35 U.S.C. 102(e) as being anticipated by Katz et al (U.S. 2002/0111317 A1) (Date of Filing is **September 24, 2001**).

For ***claim 1-9 and 17-19***, Katz et al (see entire document) discloses "recombinant DNA compounds that encode the proteins required to produce sixteen-member macrolides as well as proteins that further modify these macrolides are provided [including Applicants' elected narbomycin]. In one embodiment, recombinant DNA compounds that encode portions of these proteins are provided. In another aspect of the present invention, recombinant DNA compounds that encode a hybrid protein that is the product of one or more PKS genes are provided wherein the hybrid protein encodes all or portion of a protein

involved in the biosynthesis of sixteen-membered macrolide. In one embodiment, the recombinant DNA compounds of the invention are recombinant DNA cloning vectors that facilitate manipulation of the coding sequences or recombinant DNA expression vectors that code for the expression of one or more of the proteins of the invention in recombinant host cells. In another aspect of the present invention, recombinant host cells are provided for the expression of PKS genes" (see Katz et al, page 2, column 2, paragraph 4; see also pages 8-27 showing specific examples; see especially, page 11, Table 1, Picromycin entry disclosing Applicants' elected narbomycin and pikromycin) (Please note that the reference for narbomycin by Xue et al was incorporated by reference into Katz et al, see page 10, column 2, paragraph 2, "All of these publications are incorporated herein by reference ... The domains, modules and subunits that are described by the PKS genes listed in Table 1 as well as the genes for polyketide modification or tailoring enzymes are among those that can be used in the practice of the present invention), which anticipates claims 1-9 and 17-19.

Contact Information

21. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jon D Epperson whose telephone number is (703) 308-2423. The examiner can normally be reached Monday-Friday from 9:00 to 5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on (703) 306-3217. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9306 for regular communications and (703) 872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-2439.

Jon D. Epperson, Ph.D.
August 13, 2003

BENNETT CELSA
PRIMARY EXAMINER